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University of Turku

Faculty of Science and Engineering
Department of Biology
Section of Ecology
Doctoral programme in Biology, Geography and Geology (BGG)

Supervised by

Professor, Jon E. Brommer
Department of Biology
University of Turku
Turku, Finland

Reviewed by

Post-doctoral Fellow, Mark C. Mainwaring
Division of Biological Sciences
University of Montana
Missoula, USA

Senior Lecturer, Markus Öst
Environmental and Marine Biology
Åbo Akademi University
Turku, Finland

Opponent

Associate Professor, D. Charles Deeming
School of Life Sciences
University of Lincoln
Lincoln, UK

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We are, if nothing else, builders,
and that leads us to admire other builders.

– Mike Hansell in ”Built by animals –
the natural history of animal architecture”

UNIVERSITY OF TURKU
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PAULIINA JÄRVINEN: Causes and consequences of variation in blue tit nest construction
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ABSTRACT

Species across taxa and the vast majority of birds build nests for reproduction. The bird nest functions as a receptacle for the eggs and young and as such determines successful reproduction. Through nest construction birds are able to extend control over the environment and to create a suitable microclimate for incubation and nestling development. Nests of the order *Passeriformes* are the most varied out of any group of birds. Intriguingly, variation in size and components can be observed both between and within species nest design. In the present thesis, I investigated the causes and consequences of the variation in nest size and components in a population of blue tits, *Cyanistes caeruleus*, in Southern Finland.

In chapter **I**, I studied the effects of an experimental manipulation of nest size (height of the nest material within the nest box) on different breeding parameters related to nestling growth and female somatic condition. Variation in nest size is hypothesised to relate to varying requirements for insulation due to differences in latitude or ambient temperature. In line with the hypothesis, I expected the increased insulatory capacity of the experimentally enlarged nests to be reflected in nestling development and/ or in improved female somatic condition due to a reduced requirement for nest attendance. Blue tit chicks are ectothermic until they reach 7-8 days of age and rely mainly on the female's body heat until then to maintain their body temperature. I found little support for the thermoregulation hypothesis as only the nestling tail length increased significantly in experimentally enlarged nests. Other studies have found that instead of an increase in nest height, which is mostly contributed to by the base layer of moss and grass, more important to insulation are the mass and material components of the nest cup lining.

In chapters **II**-**IV**, my objective was to determine the evolutionary potential of variation in nest construction. In chapter **II**, I regarded the nest size and material components as potential invididual traits, whereby I studied the between-individual consistency (repeatability) and heritability in these nest characteristics by means of quantitative genetics. Because repeatability sets the upper limit for heritability, it provides a reasonable stepping-stone for further analysis of heritability. I found that both nest size and the proportion of feathers were repeatable female traits, and by further analysis, discovered that the latter was also a modestly heritable (albeit not statistically significant) trait limited to females in blue tits.

In chapter **III**, I studied nest ornamentation in blue tits and the behavioural correlations between the different nest traits. Nest ornaments are conspicuous material placed on top of the nest, typically feathers (which I focused on here), but can also be flower petals, human-made material, colourful plastics or other unusual material. I found that nest ornamentation was a repeatable behaviour in female blue tits, which contradicts with other studies conducted in a different population of blue tits, in which feather ornaments were provided by males, not females. I also found that nest ornamentation and the proportion of feathers in the nest formed an extended phenotype syndrome, a term which was introduced here. Instead of using direct observations of individual behaviour, which are required in studying behavioural syndromes, I studied the behaviours by proxy, using measurements of nests as physical remnants of nest construction behaviour. A syndrome in nest building implies that blue tits are restricted in flexibility in their response to change in their nesting environment.

In chapter **IV**, I studied selection in blue tit nest construction. I investigated whether nest characteristics have consequences on female fitness by studying her reproductive success in terms of nestling survival and offspring recruitment. I found that although nest characteristics were not related to nestling survival, the propensity to use feathers in the nest significantly increased recruitment probability. This is a significant finding and in combination with our other results (**II**) provides first evidence of selection in avian nest construction. Experimental studies are warranted to determine the selective advantage provided by the nest feather content, for it appears to become beneficial only after the offspring have left the nest.

In this thesis, I investigated some of the causes and consequences of variation in blue tit nest construction. By means of an experimental set-up, I discovered that the height of the nest material is likely to have only a negligible effect on nest thermoregulation (**I**). I found that nest ornamentation, nest height and the proportion of feathers in the nest are repeatable female traits (**II**, **III**), and provided first pedigree-wide quantitative genetic evidence of heritability in avian nest construction (**II**). Moreover, I discovered that the modestly heritable trait in female blue tits has consequences for female fitness and is thus subject to selection (**IV**). However, my finding that the proportion of feathers forms an extended phenotype syndrome with nest ornamentation implies a limitation in the range of adaptive response to environmental change (**IV**).

KEYWORDS: Nest construction, bird nest, nest trait, blue tit, Cyanistes caeruleus, extended phenotype, extended phenotype syndrome, repeatability, heritability, selection, nest size, nest design, nest ornament, feathers

TURUN YLIOPISTO
Luonnontieteiden ja tekniikan tiedekunta
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TIIVISTELMÄ

Useat eläinlajit ja miltei kaikki linnut rakentavat pesiä lisääntymiseen. Linnunpesä suojaa munia ja poikasia sääolosuhteilta sekä ympäristön uhilta ja määrittää näin lisääntymismenestystä. Rakentamalla pesän linnut luovat sopivat olosuhteet haudonnalle ja jälkeläisten kehitykselle. Pesien koot ja kompositiot vaihtelevat niin lajiensisäisesti kuin lajien välillä. Varpuslintujen (*Passeriformes*) pesissä on kaikista lintulahkoista eniten vaihtelua. Väitöskirjassani tutkin sinitiaisten (*Cyanistes caeruleus*) pesien koko- ja materiaalivaihtelun syitä ja seurauksia luonnonpopulaatiossa Etelä-Suomessa.

Ensimmäisessä osajulkaisussa tutkin kokeellisen pesänkorkeusmanipulaation (pesän korkeus linnunpöntön sisällä) vaikutuksia poikasten kasvuun ja naaraiden somaattiseen kuntoon. Pesien koonvaihtelun on arveltu liittyvän erilaisiin pesäneristystarpeisiin, jotka johtuvat maantieteellisen sijainnin tai lämpötilan eroista. Tämän hypoteesin perusteella oletin, että kokeellisesti suurennettu pesä loisi suotuisammat olosuhteet poikasten kehitykselle ja/ tai edellyttäisi vähemmän naaraan läsnäoloa pesässä. Sinitiaisten poikaset ovat vaihtolämpöisiä ensimmäisen elinviikkonsa ajan, jolloin niiden kehonlämmön ylläpitäminen edellyttää vanhemman, yleensä naaraan, läsnäoloa. Koe vaikutti ainoastaan poikasten pyrstösulkien pituuteen, millä ei oletettavasti ole merkittävää vaikutusta yksilön kelpoisuuteen, sillä pyrstön pituuden tehtävä on lennon hienosäätö. Tulokseni eivät olennaisesti tukeneet hypoteesiä, jonka mukaan suurempi pesä olisi eristysominaisuuksiltaan pientä parempi. Muiden tutkimusten mukaan pesän pintakerroksen paksuus ja materiaalit vaikuttavat enemmän pesän eristysominaisuuksiin kuin pesän korkeus, josta suurin osa koostuu sammaleisesta pohjakerroksesta, jolla on lähinnä rakenteellinen tehtävä.

Osajulkaisuissa **II**-**IV** tutkin pesänrakennuksen vaihtelun evolutiivista potentiaalia. Osajulkaisussa **II** käsittelin kvantitatiivisen genetiikan keinoin pesän kokoa ja materiaalisisältöä mahdollisina yksilöllisinä piirteinä tutkimalla näiden ominaisuuksien yksilöiden välistä toistuvuutta (*repeatability*) ja periytyvyyttä. Koska toistuvuus määrittää periytyvyyden ylärajan, siitä on perusteltua aloittaa tutkittaessa piirteen tai käyttäytymisen periytyvyyttä. Havaitsin, että sekä pesän koko että höyhenten osuus pesämateriaaleista olivat toistuvia (eli yksilöllisiä) piirteitä sinitiaisnaarailla. Lisätutkimuksen myötä selvitin, että höyhenten osuus pesästä on lisäksi lievästi periytyvä naaraisiin rajoittunut piirre.

Kolmannessa osajulkaisussa tutkin sinitiaisten pesänkoristelua sekä yksilöllisten pesänpiirteiden välisiä keskinäisiä korrelaatioita. Keskityin tässä osajulkaisussa pesäkoristeisiin, jotka ovat pesän päälle aseteltuja näyttäviä sulkia ja höyheniä, mutta koristeet voivat olla myös muuta materiaalia, kuten kukkien terälehtiä, keinotekoista materiaalia, värikästä muovia tai muuta harvoin tavattua pesämateriaalia. Havaitsin, että pesänkoristelu on toistuva piirre sinitiaisnaarailla, mikä on ristiriidassa aiemmin julkaistujen tutkimusten kanssa, joiden mukaan sinitiaiskoiraat koristelevat pesän sulilla. Lisäksi havaitsin, että pesänkoristelu ja pesän höyhenten osuus korreloivat keskenään ja muodostivat yhdessä käyttäytymissyndrooman, jota kutsuimme tässä yhteydessä laajennetuksi fenotyyppisyndroomaksi. Käyttäytymissyndrooman tutkiminen edellyttää suoria havaintoja yksilöiden käyttäytymisestä. Korvasimme suorat havainnot pesänrakennuskäyttäytymisestä tutkimalla sen jäänteitä, eli pesien piirteitä. Havaittu pesänrakennussyndrooma (eli keskenään korreloivat pesänpiirteet) vihjaa, että sinitiaisten vaste pesimisympäristön muutokseen voi olla rajallinen, ainakin jos pesänpiirteet korreloivat fenotyypin lisäksi myös geneettisellä tasolla.

Osajulkaisussa **IV** tutkin valintaa sinitiaisten pesänrakennuskäyttäytymisessä. Selvitin, onko pesänpiirteillä yhteyksiä kelpoisuuteen tutkimalla naaraan lisääntymismenestystä. Havaitsin, että vaikka pesänpiirteet eivät vaikuttaneet pesäpoikasten todennäköisyyteen selviytyä, höyhenten osuus pesässä lisäsi merkitsevästi poikasten rekrytointitodennäköisyyttä lisääntymispopulaatioon. Tämä on merkittävä löytö, sillä yhdistettynä muihin tuloksiimme (**II**), se on ensimmäinen todiste valinnan vaikutuksesta lintujen pesänrakennuskäyttäytymiseen. Kokeelliset tutkimukset ovat tarpeen selvittämään pesähöyhenten aiheuttama suotuisa vaikutus, josta on hyötyä vasta sen jälkeen, kun poikaset lähtevät pesästä.

Väitöskirjassani tutkin sinitiaisten pesien vaihtelun syitä ja seurauksia. Kokeellisen tutkimuksen avulla havaitsin, että pesän korkeudella on vähäinen vaikutus pesän lämmönsäätelyominaisuuksiin (**I**). Havaitsin, että pesänkoristelu, pesän korkeus sekä höyhenten osuus pesästä ovat toistuvia piirteitä naarailla (**II**, **III**), ja löysin kvantitatiivisen genetiikan keinoin todisteita pesänrakennuskäyttäytymisen periytyvyydestä (**II**). Lisäksi havaitsin, että höyhenten osuus pesässä on naaraan piirre, jolla on periytyvyyden lisäksi vaikutuksia tämän kelpoisuuteen, mikä tarkoittaa, että se on valinnan kohteena (**IV**).

ASIASANAT: Pesänrakennus, linnunpesä, pesäpiirre, sinitiainen, Cyanistes caeruleus, laajennettu fenotyyppi, laajennettu fenotyyppisyndrooma, toistuvuus, perinnöllisyys, valinta, pesän koko, pesäaines, pesäkoriste, höyhenet

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List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Järvinen, P. H., Kluen, E., Tiiri, M., & Brommer, J. E. (2017). Experimental manipulation of Blue Tit nest height does not support the thermoregulation hypothesis. *Ornis Fennica*, *94*(2), 82-92.

II Järvinen, P., Kluen, E., & Brommer, J. E. (2017). Low heritability of nest construction in a wild bird. *Biology letters*, *13*(10), 20170246.

III Järvinen, P., & Brommer, J. E. Nest ornaments and feather composition form an extended phenotype syndrome in a wild bird. *Submitted Manuscript.*

IV Järvinen, P., & Brommer, J. E. Selection on nest characteristics and ornamentation in a wild bird population. *Submitted manuscript*.

Author contributions to the original publications:

	I	II	III	IV
Original idea	JEB, EK	JEB, EK	JEB, PJ	JEB
Data collection	JEB, EK, PJ	JEB, EK, PJ	JEB, PJ	JEB, PJ
Statistical analyses	MT, PJ, JEB	JEB, PJ	PJ, JEB	PJ
Writing	PJ, EK, MT, JEB	JEB, PJ, EK	PJ, JEB	PJ, JEB

PJ = Pauliina Järvinen, JEB = Jon E. Brommer, EK = Edward Kluen, MT = Maiju Tiiri

1 Introduction

1.1 Nest construction

Nest construction is a widely spread reproductive activity within the animal kingdom. Most birds and mammals build nests, as do many insects, arachnids, reptiles, amphibians, fish and even crustaceans (Hansell 2000; 2007). The majority of studies investigating nest construction behaviour focus on birds, but also invertebrate, mammal, fish, amphibian and reptile nests have been featured in research (Barber 2013).

In birds, nest construction is an integral part of reproduction. By building nests, birds extend control over the environment and create a suitable microclimate for their offspring to develop in. In fact, nest building has allowed birds to inhabit highly diverse habitats (Collias 1997) and may have protected bird ancestry when the non-avian dinosaurs went extinct (Hansell 2000). Avian nest types are varied and range from scrape nests that are mere indentations on the ground of most shorebirds, gallinaceous birds, and some birds of prey among others (Fang *et al.* 2018) to elaborate woven constructs by Eurasian penduline tits (*Remiz pendulinus;* Pogány and Székely 2007) and to the enormous communal nests of sociable weavers (*Philetairus socius;* Maclean 1973) and Montezuma oropendolas (*Psarocolius montezuma;* Howell 1964).

Nests of the order *Passeriformes* (hereafter referred to as passerines) are the most diverse and elaborate of all bird nests. Passerine nests can be divided into open cup-nests, domed nests and cavity nests. While open cup-nests are the most common type of a passerine nest and also the least energy expensive to construct, they provide less protection than domed nests and nests built inside cavities (Collias 1997). Cavity nesters are further constrained by the limited availability of natural cavities (Collias 1997). Hence, species that build open cup-, domed and cavity nests are exposed to different selection pressures. For this reason, the focus of this thesis is on cavity nests, while research on open-cup nests is cited whenever relevant.

Cavity nests are either cup-nests or domed nests that are built inside a cavity, a burrow or a nest box, but domed nests can also be freestanding (Collias 1997). These two types of nests can be especially complex structures and building them

entails costs and trade-offs in terms of time, energy and predation risk. The time spent in construction must be traded-off against other fitness-related behaviours (e.g. foraging) (Mainwaring and Hartley 2009) and gathering the nest materials exposes the builder to predators (Collias and Collias 1984). In return, nests shelter the parents, eggs and offspring against predators and environmental fluctuations (Skowron and Kern 1980, Hansell 2000) and may also function as a sexual signal. Because nest construction is a costly behaviour (Mainwaring and Hartley 2009), its end product – the nest – is an honest signal of the builder's condition and willingness to invest in the current reproductive event (Moreno 2012).

1.2 Within-species variation in avian nest design

Bird nests contain a tremendous amount of variation. Most intriguingly, variation in nest design is not restricted to species-level differences but exist also within species. Several hypotheses have been presented to explain the within-species variation in nest design. These hypotheses revolve around thermoregulation, sexual signalling, ectoparasite, bacteria, and predator avoidance, and also nest luminance and flooding avoidance. The thermoregulation hypothesis suggests that larger nests have a higher capacity for heat retention (Collias and Collias 1984). The sexual signalling hypothesis posits that due to the costs related to nest construction, nests can function as honest signals of the builders' fitness (Jose *et al.* 1998; Moreno 2012). According to the anti-parasite and anti-bacteria hypotheses, certain nest materials are incorporated due to their parasite repelling or antibacterial attributes (López-Rull and Macías Garcia 2015). The hypothesis on flooding avoidance considers a larger nest size and the choice of nest material with reduced water-retention capacity (i.e. fast-drying) to benefit in wet and rainy conditions (Wesołowski *et al.* 2002; Biddle *et al.* 2019). The hypothesis on predator avoidance relates especially to open nests, in which certain materials can increase or decrease their conspicuousness to predators (Møller 1987; Bailey *et al.* 2015). In cavity-nesting birds, a larger nest size reduces the distance between the nest cup and the entrance hole, thereby placing the nestlings at a higher risk of predation (Kaliński *et al.* 2014). The hypothesis on nest illumination also considers the nest size within a cavity and suggests that the height of the nest material can vary spatially depending on the level of light available inside the cavity. Developing embryos require a certain amount of natural light e.g. for normal cognitive development (Maurer *et al.* 2011), but exposure to urban light pollution is detrimental to both parents and nestlings (Holveck *et al.* 2019).

The avian nest represents an evolutionary compromise between several benefits and constraints. Thus, the different selection pressures described above never function in isolation. The strength and direction of selection can vary both in time

and space, which is in turn reflected as variation in nest size and design (reviewed by Mainwaring 2017). For example, in blue tits (*Cyanistes caeruleus)* and great tits (*Parus major*), nests of greater height and mass signal of the builders' fitness (reviewed by Moreno 2012; Tomás *et al.* 2013) and can keep the nests dry in case of a flooding event (Wesołowski *et al.* 2002). Large nests also provide thermoregulatory benefits (Collias and Collias 1984; Deeming *et al.* 2012; Mainwaring *et al.* 2012; 2014). In contrast, higher nests can attract more ectoparasites (Eeva *et al.* 1994), increase the risk of nest predation (Kalínski *et al.* 2014) and may expose the birds to light pollution (Holveck *et al.* 2019).

Besides in size, nests vary in material components. Blue tits and spotless starlings (*Sturnus unicolor*), for example, incorporate aromatic plant matter into the nest (Mennerat *et al.* 2009a; Polo and Veiga 2006). For spotless starlings, plants function as a sexual signal, which is shown by male starlings with a higher testosterone level carrying more plants to the nest (Polo and Veiga 2006). For blue tits, the herbs provide anti-bacterial benefits, which is positively reflected in nestling development (Mennerat *et al.* 2009b). Urban birds, on the other hand, may opt for an urban solution and have been reported to use cigarette butts in place of aromatic plants (Suárez-Rodríguez *et al.* 2013). Compared to other nesting material, feathers have a superior insulation capacity (Hilton *et al.* 2004) but heat retention is not their sole function in the nest. Feathers can be used to repel nest ectoparasites (López-Rull and Macías Garcia 2015; but see Mainwaring *et al.* 2016), mitigate the adversary effects of pathogens (Soler *et al.* 2012), and function in sexual signalling (Veiga and Polo 2005; Polo and Veiga 2006). Female spotless starlings bring feathers that they have gathered from the environment to the nest to reciprocate the aromatic plants brought by the males (Polo and Veiga 2006). These are mostly primary feathers with a reduced capacity for insulation and placed apart from the nest cup in a position that allows maximum visibility from the nest entrance (Veiga and Polo 2005). They are arranged non-randomly so that the side of the feather that provides the maximum UV reflectance is faced upwards: some feathers (of spotless starlings and wood pigeons, *Columba palumbus*) are placed the reverse side pointing upwards, yet in other feathers (of jays, *Garrulus glandarius*) the obverse side is made visible. The feathers of the azure-winged magpies (*Cyanopica cyana*), on the contrary, display similar reflectance values on both sides and are thus positioned randomly on either side (Veiga and Polo 2005).

Conspicuous feathers, such as those described above, and other striking materials gathered from the surroundings that are placed on top of the nest are sometimes referred to as nest ornaments for their putative role in sexual signalling (Sanz and García-Navas 2011; García-Navas *et al.* 2013). Black kites (*Milvus migrans*) add white bits of plastic on their nests, which convey information about their fitness and competitive ability to their conspecifics. An experimental addition

of the material significantly reduced the trespassing by competitors into the builders' territory (Sergio *et al.* 2011). In blue tits, females are known to build the nests alone (Cramp and Perrins 1993), although males in one population have been reported to carry feathers to the nest (Sanz and García-Navas 2011). Subsequent work in the same population studied the effects of experimentally supplemented feathers: as the feathers were removed, the social males in these nests reduced their rate of provisioning. Apparently the social males considered that the supplemental feathers were an indication of a cuckolding attempt by extra-pair males and they responded accordingly by reducing their investment in the offspring that they had potentially not sired (García-Navas *et al*. 2013).

1.3 Female sexual signalling and nest construction

Nest characteristics, such as nest size or material composition, can relate information on the builder's quality and reproductive investment to the mate of the builder (Moreno 2012). Experimental manipulations of nest features have shown that the mates respond to these signals by adjusting their own investment in reproduction (e.g. Soler *et al.* 1996; 2001; Polo and Veiga 2006; Sanz and García-Navas 2011; Tomás *et al.* 2013). It can thus be concluded that nests have the potential to act as secondary sexual signals of the builder.

Historically, studies on sexual signalling have mostly revolved around males to the extent that sexually selected ornaments were often defined in terms of sexual dimorphism (Kraaijeveld *et al.* 2007). Or, in taxa where both sexes carry ornaments – such as antlers in ungulates or colourful plumages in hummingbirds – female ornamentation was assumed to be non-adaptive correlative effects of selection on males (Amundsen 2000). With the development of field studies came the recognition of between-female competition and its repercussion on female signalling (Clutton-Brock and Huchard 2013), which was further increased by experimental studies (Amundsen 2000). Today, it is known that sexual signalling in females has developed in a substantial number of species (Kraaijeveld *et al.* 2007). Irrespective of sex, secondary sexual signals are likely to evolve when the competition of resources for reproduction is intense, for example, when the population density is high or the operational sex ratio (the ratio of sexually receptive males to females) is biased, which increases intrasex competition in the sex it is biased towards (Emleng and Oring 1977).

Females can gain fitness benefits through signalling by attracting high quality mates who provide good genes and increased viability for the offspring (based on the handicap principle, Zahavi 1975), and by attracting good fathers whose investment in paternal care may both increase offspring survival and allow the female to redirect resources from maternal care to self-maintenance (Hoelzer

1989). However, females that signal via nest construction are likely to accrue benefits through differential allocation (Burley 1986; Sheldon 2000) instead of mate competition because pairs have already formed and mated before nest building (Moreno 2012). Males may modulate their reproductive investment in terms of perceived female effort, which is reflected in paternal care and nest defence. For example, Tomás *et al.* (2013) provided experimental evidence that blue tit males adjust their risk taking in response to nest size and to the presence of aromatic plants in the nest. After a human had visited the nest, males of experimentally enlarged and control nests and nests with experimentally added aromatic plants were more likely to enter the nest box before females (Tomás *et al* 2013). Previously, Tomás *et al.* (2006) had shown that nest size is related to female health status as female blue tits infected with Trypanosoma hemoparasites built smaller nests and their blood immunoglobin levels were negatively related to the nest size. Another study on blue tits and one conducted on their close relative, the great tit, also provided evidence that female health status is linked to her nest size (Broggi and Senar 2009: Mainwaring and Hartley 2009). According to Mainwaring and Hartley (2009), supplementary fed blue tits built heavier nests; while Broggi and Senar (2009) showed that the more brightly coloured female great tits built larger nests (Broggi and Senar 2009). In spotless starlings, the amount of feathers that females carried to their nests increased in response to experimentally supplemented aromatic plants, which is nest material that starling males provide (Polo and Veiga 2006). The amount of feathers was positively related to the female body condition as proxied by two correlating variables, the lay date and experience in breeding (Polo and Veiga 2006). A later study showed that experimentally added feathers reduced nestling mortality, thereby providing further evidence of sexual selection functioning on a female trait (Veiga and Polo 2011). The most recent study found that increased competition is the likely ultimate cause of nest decoration in spotless starlings as pairs that bred in a high-density area added more feathers and aromatic plants compared to pairs nesting in a low-density area (Rubalcaba *et al.* 2017).

1.4 Evolutionary consequences of nest variation

Most of the hypotheses explaining within-species nest variation described in the previous section presume that nest construction is adaptive. Adaptation is a genetic change driven by selective forces that act on genetic variation in a trait. Thus, the ability to adapt requires that the trait in question is both heritable and has consequences on fitness. Not all variation, however, is caused by adaptation but may be a manifestation of phenotypic plasticity, which is an organism's existing potential to respond to environmental variation. It is important to tease apart these

two sources of variation because only variable genetic material in combination with a selective agent enables adjustment to novel circumstances and at a rate that is determined by the strength of selection. However, forays into quantitative genetics of avian nest construction have been practically non-existent and thus reliable estimates of the adaptive potential of nest construction is severely lacking.

Repeatability, a measure of consistency in individual behaviour, can be described as the proportion of observed total phenotypic variation that is explained by variation between individuals (Falconer and Mackay 1996). Several nest elements have been found to be repeatable either within or across breeding seasons, which indicates that they could contain a genetic component. Between-invididual variation in a trait is the function of a genetic component combined with the environmental influence, indicating that repeatability sets an upper limit of heritability (Falconer and Mackay 1996). Studies on the repeatability of nest construction primarily focus on birds, but nest construction has been found repeatable also in some fishes (Rushbrook *et al.* 2008; Japoshvili *et al.* 2012). Some examples of repeatability in avian nest construction include consistent preferences for nesting materials in blue tits (Mennerat *et al.* 2009b), pied flycatchers (*Ficedula hypoleuca*; Briggs and Mainwaring 2019) and common starlings (*Sturnus vulgaris*: Gwinner and Berger 2008). In addition, southern masked weaverbirds (*Ploceus velatus*) show repeatability in nest size within season (Walsh *et al.* 2010) and blue tits across seasons (O'Neill *et al.* 2018).

Besides our work (presented in chapter **II**), only Møller (2006) has provided evidence of heritable nest construction in birds. Møller reported in a study that spanned from 1977 to 2003 that in barn swallows (*Hirundo rustica*), where males are responsible for nest building, sexual selection by females shifted from nest size, which originally functioned as the sexually selected extended phenotype of the male, to another secondary sexual character, the male tail length. Møller (2006) was able to observe a simultaneous reduction in nest size and an increase in the male tail length and found that these two traits were genetically highly correlated. Because males with shorter tails built larger nests and provided more parental care, the shift in selection towards longer tails reduced the nests to one third of their original size in only 26 years (Møller 2006). O'Neill *et al.* (2018) found nest size to be a consistent but not a heritable individual trait in female blue tits. Both Møller (2006) and O'Neill *et al.* (2018) employed the method of parent-offspring resemblance to study heritability in nest construction instead of the more robust quantitative genetic method of the 'animal model' (Kruuk 2004; Kruuk and Hadfield 2007; Åkesson *et al.* 2008). In the parent-offspring resemblance, heritability is estimated according to trait correlations between the offspring and the biological parent, exposing the estimates to the potential bias of cultural inheritance, where the offspring simply replicate their natal nests (Kruuk and

Hadfield 2007). O'Neill *et al.* (2018) was able to eliminate this bias by conducting a cross-fostering experiment where the nest sizes of the offpsring were compared to those of both biological and foster mothers. The animal model uses trait correlations between all relatives in the pedigree and is thus generally less vulnerable to the confounding effect of cultural inheritance (Kruuk 2004; Kruuk and Hadfield 2007; Åkesson *et al.* 2008). Because the animal model relies on pedigree-derived estimates of relatedness, its application requires long datasets. Obtaining pedigree data in a natural population requires long-term effort, which likely explains the scarcity of quantitative genetic studies in nest construction.

1.5 Aims of the thesis

My objective in this thesis was to study intraspecific variation in nests built by a population of wild blue tits. In chapter **I**, I focused on the variation in nest size and approached it from the thermoregulatory aspect. In line with the thermoregulation hypothesis (Collias and Collias 1984), I expected experimentally enlarged nests to provide a thermoregulatory benefit that would be displayed either in faster nestling development or an improved female somatic condition. I assumed that the latter would reflect a reduction in the trade-off between female nest attendance and foraging. In chapters **II**-**IV**, I focused on the evolutionary potential in blue tit nest construction. I studied repeatability (**II**-**III**) and heritability (**II**) in nest size and material components. Because females build the nests and choose the nesting sites in blue tits (Cramp and Perrins 1993), I expected the female identity or her physical attributes to be related to the variation in the nest characteristics (**II**). But if the males were responsible for the nest feather contents, as has been reported in a population of blue tits in Spain (Sanz and García-Navas 2011; García-Navas *et al.* 2013), I expected the male identity to explain variation in the proportion of feathers in the nest (**II**) and/ or in the number of feather nest ornaments (**III**). In chapter **III**, I studied both the individual consistency in nest ornamentation behaviour, i.e. placing of conspicuous feathers and other material on top of the nest potentially in sexual signalling purpose, and covariation between the repeatable nest traits. I expected that if the traits were correlated, they would form a nest construction (behavioural) syndrome, which would indicate that the traits might not evolve in isolation. If the traits were not correlated, I expected them to be able to respond to selection individually and thus with greater flexibility. Lastly, I studied the fitness consequences of nest characteristics in aim to determine the selective potential in blue tit nest construction (**IV**). I expected the probability for nestling survival and/ or offspring recruitment to vary in response to the nest characteristics if they were subject to selection.

2 Materials and Methods

2.1 Study species

The Eurasian blue tit (*Cyanistes caeruleus*, hereafter blue tit) is a small passerine bird species that is spread throughout the Western Palearctic (Stenning 2018). The conservation status of the species is 'least concern' with a population trend predicted to increase (Birdlife International 2017). The blue tit is one of the most common model species used in research on avian ecology due to its abundance and affinity to breed in man-made nest boxes. The blue tit is a socially monogamous cavity-nesting species that has one to two broods per breeding season, which begins in mid to late April and is finished by early to mid July in southern Finland. Females build the nest alone (but males may provide feathers in some populations: Sanz and García-Navas 2011; García-Navas *et al.* 2013) and lays between 6 to 14 eggs. The incubation period lasts approximately 12 days. The timing of the first broods (typically hatch between late May to early June) coincides with the abundance of *Lepidoptera* larvae feeding on birch leaves, which is the main food source for the nestlings (Cramp and Perrins 1993; Nour *et al.* 1998). The chicks hatch weighing around 1 gram and reach adult weight of about 12 grams by the time they fledge, which usually takes place on day 18-20 after hatching.

2.1.1 The blue tit nest

The blue tit nest consists of a base layer and a lining. The base, or the structural layer, is made of moss and dry grass and the lining, which is the insulatory layer, of grass, hair, fur, wool and feathers (Britt and Deeming 2011, Mainwaring and Hartley 2009, Fig. 1). Other typical nest components include bark, wool, pine needles, lichen and fresh plant matter. Small mammal fur, flower petals and human-made materials such as insulation wool are also occasionally incorporated (Fig. 2).

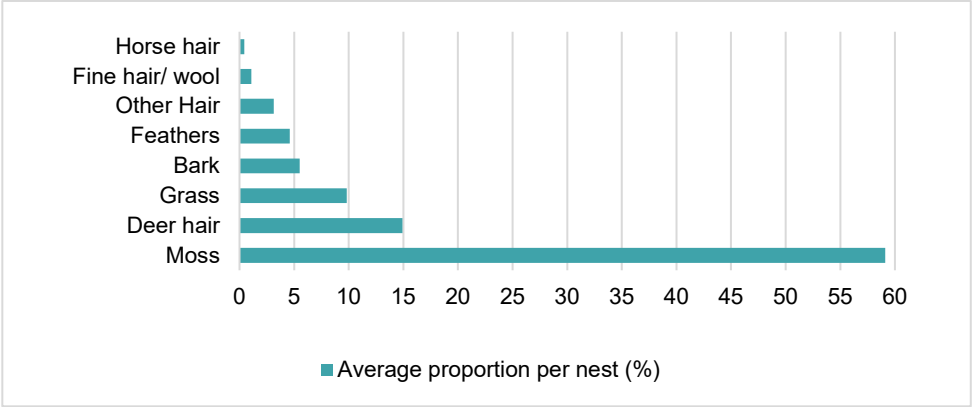

Figure 1 The components of blue tit nests that were observed in the highest proportions in our study population between 2008-2019. The figure portrays a typical nest out of which the base layer formed approx. 60-70 % of the total nest volume and consisted of moss interlaced with grass. The lining formed the top layer and consisted of mostly hair and feathers. Bark was incorporated both in the base layer and the lining. The nest components not included here (see Fig. 2) each made up maximum of 0.2 % on average of total nest components and was found mainly in the lining.

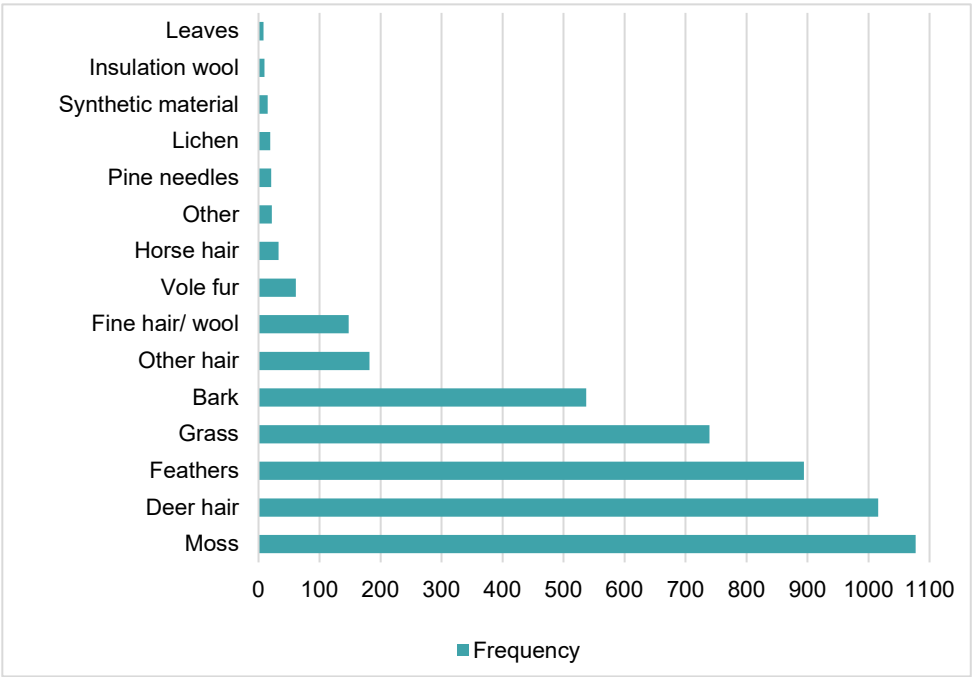

Figure 2 The frequence of nest components observed in 1082 blue tit nests within our study site in 2008-2019.

2.2 Study area

The data used in this thesis (2006-2019) was collected as part of a long-term project that began in 2005 near the city of Tammisaari, in Southwestern Finland (60°01' N, 23°31' E). The study site consists of approximately 10 km^2 of mostly commercially used mixed boreal forest interspersed with farmland (Fig. 3) and has contained between 319 and 470 nest boxes depending on the year. The greatest variation in the nest box numbers was due to clearcutting, which led to a relocation of nest boxes to other areas. Out of the total number of the nest boxes, approximately a 100 were used by blue tits per season. The nest boxes were located in areas of forest with higher than average density of birch trees. In addition to downy birch (*Betula pubescens*) and silver birch (*Betula pendula*), the woods in the area mostly consist of Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*).

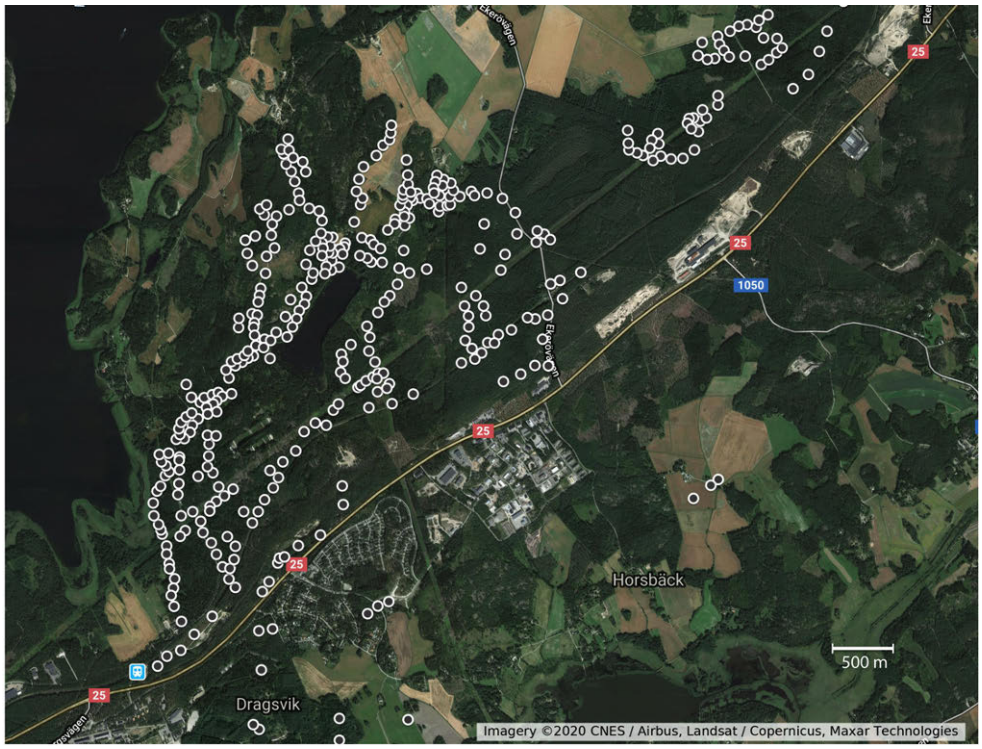

Figure 3 A satellite map image of the study site. The circles mark the locations of the nest boxes in 2010. (Source: Google maps 2020)

2.3 Data collection

2.3.1 Nest box

The nest boxes were made of untreated spruce with an openable lid for roof (W x H x D = 125 mm x 200mm x 81 mm, bottom thickness = 22mm; bottom surface area 125 x 81 mm = 101 cm^2; bottom-to-hole distance = 170 mm). They were attached to trees at about 150 cm height to allow for easy access. The entrance hole was 26 mm in diameter to allow only blue tits and coal tits (*Periparus ater*) to enter (Dhondt and Eyckerman 1980). Non-bird species also take advantage of the nest boxes and these include e.g. wasps, bumble bees, bats, slugs and various arthropods (own observation).

It is to be noted that while providing nest boxes in field studies is both a common and highly advantageous practice (Lambrecths *et al.* 2010), it nevertheless comes with disadvantages. The benefits related to the use of artificial nest boxes can also be their downside. For example, the provisioning of nest boxes have a tendency to generate a locally increased population size, which allows a healthy sample size but it also leads birds to breeding at higher densities and thereby manipulates their breeding conditions. Higher population densities may increase intraspecific competition for reproductive resources and reduce the rate of nest predation (Lambrecths *et al.* 2010). The provisioned nest boxes are often maintained, replaced and cleaned of old nest material after each breeding season and their inhabitants are thus likely to suffer from fewer ectoparasites than conspecifics breeding in natural cavities. A reduction in confounding factors, such as nest predation or ectoparasite load, as well as an increased availability of cavities to breed in, expose the study population to breeding conditions and environmental pressures that are somewhat distanced to those of their conspecifics breeding in natural cavities. These issues are particularly important to consider when comparing the results of different nest box studies, due to the variation in nest box protocols as well as the nest box types and sizes adopted by different researchers.

2.3.2 Nest height (I-IV)

The height of the nest material (hereafter nest height), that is, the distance between the floor of the nest box and the top of the nest, was measured when nestlings were two days old, except in 2011 when the nest height was not measured. The nest height was measured with 5 mm accuracy in all four corners with a knitting needle fitted with millimetre-paper (**I**) or an electronic sliding calliper (**II**-**IV**). The average of these values were used as the measure of the nest height.

2.3.3 Nest materials (II-IV)

The nest was temporarily removed from the nest box and the proportions of different nest material contents were estimated by eye in 2008–2018 (**II-IV**) when the nestlings were two days old (except in 2011 when the material contents where scored when the nestlings were 5-6 days old).

In 2006–2018, the nests were photographed from above and I was solely responsible for recording of the nest ornaments from the images to reduce inter-observer variability (**III-IV**).

2.3.4 Nest height manipulation and cross fostering (I)

In 2009 and 2010, the nest height was experimentally manipulated in 182 nests out of which 165 were successful enough to be included in the study (at least one nestling survived until day 9). On day two after hatching, the nest height was manipulated. A pair of nests with chicks of the same age and approximately the same average mass was the experimental unit. One of these nests was randomly assigned to be a 'high' and the other a 'low' nest. The original nests were removed to be microwaved to remove ectoparasites (Pitala *et al.* 2010) and replaced with an already microwaved nest. The ectoparasite removal was conducted to further remove confounding variation between nests. The high nests were manipulated to reach 11 cm in height and low nests were reduced to 5 cm in height. Depending on the treatment, moss was either added to or removed from the bottom of the nest and the top layer was left untouched. The nestlings were reciprocally cross-fostered in order to standardise the genetic and maternal effects. Both nests in the pair thus consisted of an equal number of genetic and fostered offspring of the same two families that were subjected to opposite treatments. The nestlings were ranked according to body mass and whether the heaviest was cross-fostered or not was decided at random. The cross-fostering treatment was then alternated down the size hierarchy so that an average body mass per nest was maintained.

2.3.5 Adult and nestling measurements (I, IV)

The adults were subjected to the 'adult protocol' each time they were encountered in the nest box during a hatch check or the day 2 protocol or when they were trapped for DNA extraction (when the nestlings were a minimum of 5 days of age and able to produce body heat and thus withstand a longer parental absence due to a more invasive research procedure of sampling for DNA). The procedures consisted of individual identification by metal rings, sexing on the basis of a brooding patch and aging as yearlings or ≥ 2-year-olds based on the coverts of the primary feathers (Stenning 2018) and recording the body measurements, which

included body mass (with 0.1 g precision) and tarsus length (with 0.1 mm precision) among others. The adults were weighed using a Pesola spring balance and their tarsus length was measured twice with an electronic sliding calliper. An average of these values was used as the tarsus measurement.

The nestlings were measured for body mass on day 2, 9 and 16 after hatching. On day 2 and 9 the nestlings were weighed on an electronic scale (with 0.1 g precision). On day 2, the nestlings nails were clipped in an individual-specific combination to allow identifying on day 9 when the nestlings were ringed, measured for body mass and 2-4 of their shoulder feathers were collected for DNA sampling. On day 16, the nestlings were measured in an identical manner as adults for body mass and tarsus, head, wing and tail lengths (mm).

2.4 Nest ornaments

Nest ornaments are materials that stand out of the nest due to their larger size or contrasting colour in comparison to the other nest components. Nest ornaments are typically feathers but can be e.g. flower petals or other plant matter, man-made materials like plastics (Sergio *et al.* 2011) and dyed wool, colourful pieces of string or pieces of small-mammal skin (Fig. 4). Apart from having a striking appearance, nest ornaments are determined by their location in the nest: they are placed on top of the nest and apart from the nest cup and thus ought not to function in insulation.

Figure 4 Examples of potential nest ornaments observed in the study population of blue tits: a) aromatic plants (*Rhododendron tomentosum*), b) a feather ornament, c) a feather ornament and a piece of insulation wool, d) a piece of vole skin, e) a feather ornament, and f) a piece of vole skin.

2.5 Statistical analyses

We conducted all the statistical analyses in R (2019). In chapters II and III, we used ASReml-R (v4.1.0.90, Butler 2018), a software designed for quantitative genetic analysis and it is used within the R interface.

2.5.1 Chapter I

We studied the effects of nest height manipulation on nestling growth, female body mass on day 9, and brood survival between day 2 and day 16 by means of generalised linear models (GLM; models 1.1-1.5 and 1.7) and generalised linear mixed effects models (GLMM; model 1.6) (Table 1). We assumed Gaussian errors for models 1.1-1.6 and binomial errors for model 1.7 (Table 1). We used several parameters for nestlings growth, all averaged values per brood: (1) nestling body mass on day 9; (2) nestling body mass on day 16; (3) nestling tarsus; (4) wing; and (5) tail length on day 16 (Table 1).

2.5.2 Chapter II

We analysed repeatability and heritability of nest construction traits with the animal model, which is a linear mixed effect model that uses pedigree-derived estimates of relatedness to estimate additive genetic (co)variance (Kruuk 2004). The (pruned) pedigree consisted of up to nine generations of blue tits and included 429 individuals. For 10 of these parentage was unknown. For the remaining 419 individuals whose parentage was known, the dam was identified for 417 and the sire for 403. We constructed two models, one with the proportion of feathers, which was arcsin-square root transformed to approximate a Gaussian distribution and the other with the nest height as the response variable (models 2.1 and 2.2 respectively; Table 1). In our starting models we included the ages of the female and male as fixed factorial effects because experience can affect nest construction (Muth and Healy 2011; Sergio *et al.* 2011; Bailey *et al.* 2014). As random effects, we included the year to control for temporal variation and observer identity to control for inter-observer variation. We added further random effects (models 2.1 and 2.2; Table 1) upon the starting models one at a time and tested the significance of each inclusion with likelihood ratio test (LRT). We included the nest box identity as a surrogate for the nesting site and included the female and male identitities to test for between-female and between-male variation. Because we found significant between-female variation in both models, we futher partitioned the variance into permanent environment and additive genetic effects. Permanent environment effects are reflected as within-individual variation and include, for example, maternal effects or individual plasticity (Kruuk 2004).

2.5.3 Chapter III

First, we studied the between-individual consistency in nest ornamentation behaviour in female and male blue tits. We conducted Poisson generalised linear mixed models (GLMM) with a log link (Model 3.1, Table 1). We calculated the

Table 1 All the models that were used in the statistical analyses in the thesis. Abbreviations: NH = nest height, PF = proportion of feathers in the nest, NO = numberof nest ornaments, BM = body mass, PE = permanent environment effect, A = additive genetic effect, std. = standardised to zero mean and unit standard deviation (*Z*-score), D9 = day 9 after hatching, D16 = day 16 after hatching.

CHAPTER	**I**			**II**			**III**			**IV**		
	MODEL		*Fixed/ random*	MODEL		*Fixed/ random*	MODEL		*Fixed/ random*	MODEL		*Fixed/ random*
Response variable	1.1-1.5	Parameter for nestlings growth*		2.1	PF***		3.1	NO		4.1	Fledging success/ failure	
Explanatory variable		NH manipulated Year NH*Year	*Random*		Female age** Male age**	*Fixed*		Female age** Male age**	*Fixed*		NH std. PF std. NO std. Lay date std. Female BM std. Female tarsus std.	*Fixed*
					Year Observer Nest box identity Female identity Female: PE Female: A Male identity	*Random*		Year Nest box identity Female identity Male identity	*Random*		Year Nest box identity Female identity	*Random*
Response variable	1.6	Female BM on D9		2.2	NH		3.2	NH, NO, PF		4.2	Recruitment success/ failure	
Explanatory variable		Female age**	*Fixed*		Female age** Male age**	*Fixed*		Female age** Year	*Fixed*		NH std. PF std. NO std. Lay date std.	*Fixed*

									Female BM std. Female tarsus std.	
		Female tarsus NH manipulated NH*Year	*Random*	Year Observer Nest box identity Female identity Female: PE Female: A Male identity	*Random*	Female identity Nest box identity	*Random*		Year Female identity	*Random*
Response variable	1.7	Brood survival (binomial)						4.3	Ectoparasite count	
Explanatory variable		NH manipulated Year NH*Year	*Random*						NH std. PF std. NO std. Lay date std. Female MB std. Female tarsus std.	*Fixed*
									Year Nest box identity Female identity	*Random*

* Nestlings BM on D9, nestling BM on D16, tarsus, wing, tail on D16

** 1 vs. ≥ 2 year-old

adjusted repeatability, which is the repeatability that is conditional upon the fixed effects of the model (Nakagawa and Shielzeth 2010), of nest ornamentation on the latent scale. This was computed as the ratio of the between-individual variance in nest ornamentation over the total sum of the variance components that were included in the Poisson regression.

Second, we considered the repeatable aspects of nest construction (the nest height and proportion of feathers (**II**) and nest ornamentation) as an extended phenotype syndrome. To study covariance between nest characteristics, we constructed a multivariate mixed model with nest ornaments modelled as a Poisson distributed variable and nest height and arcsin-square root transformed proportion of feathers as Gaussian distributed variables (Model 3.2; Table 1). The correlations that were required to identify a syndrome between the nest characteristics were calculated based on the estimates provided by the models.

2.5.4 Chapter IV

First, we studied blue tit nest construction as an adaptive behaviour by constructing Generalised Linear Mixed Models (GLMM) with two different metrics for reproductive success as binomial response variables: fledging success and local recruitment success (4.1 and 4.2; Table 1). Fledging success was measured as the proportion of the brood that fledged successfully out of the total clutch size in 2012-2019. Recruitment success was measured as the proportion of fledglings that was later encountered breeding within the study site. The offspring that hatched in 2012-2017 were included in the recruitment analysis to allow a minimum of two years for recruitment. We considered the fledging success to provide the upper estimate of reproductive success, while local recruitment success would provide the lower estimate. This is because of all the offspring that dispersed from the study site were naturally excluded from the analysis. Because the lay date and the geometric mean of the temperature during the incubation period and the first seven days after hatching per brood were highly correlated measures ($r < 0.8$), we included only lay date as a variable in all three models. Due to the high covariance between the female and nest box identities, we were required to exclude the nest box identity from the model for recruitment success.

Second, we studied the relationship between the nest ectoparasite abundance as a function of the nest characteristics by the means of a GLMM with a negative binomial distribution. In this model, we used the count of hen fleas (0-60) on day 16 as the response variable (Model 4.3, Table 1). As in the models for reproductive success, we were required to eliminate the temperature variable due to the high correlation with the lay date (Model 4.3, Table 1).

3 Results and discussion

3.1 Experimental manipulation of nest height

In chapter **I**, we used an experimental set-up to determine whether nest height has a thermoregulatory function. In line with the hypothesis that larger nests provide thermoregulatory benefits, we expected experimentally enlarged nests to show increased growth of nestlings compared to shallow nests, or to improve female somatic condition. We found that the nest height manipulation affected the tail length of 16-day old nestlings but did not affect any other morphometric measure (tarsus length, body mass, head size and wing length). In addition, nest height had no impact on nestling survival and did not affect female body condition. Our results do not therefore provide strong support for the thermoregulatory hypothesis.

While a number of studies in *Paridae* have found a significant inverse relationship between temperature and nest mass (e.g. Britt and Deeming 2011; Crossman *et al.* 2011), others have not (e.g. Lambrechts and Caro 2018). Our experiment focused on nest height provided mostly by the structural layer of the nest (Hansell 2000), which consists of moss and grass and as such has little insulation value (Mainwaring *et al.* 2012). Mainwaring *et al.* (2012) found that while the mass of the nest lining material was significantly positively correlated with insulation capacity, the mass of the base was not. This was further supported by their finding and that of Deeming *et al.* (2012) that the mass of the cup lining varied inversely in response to latitudinal temperature variation (Mainwaring *et al.* 2012).

Our results in combination with those listed above could suggest regional differences in the relationship between the nest size and reproductive success. However, when comparing the results of different nest box studies, it is important to note that the dimensions and construction materials of nest boxes may vary study to study and to recognise the implications this may have on the comparability of the results. For example, Møller *et al.* (2014) found that blue tits laid larger clutches in wooden than in concrete nest boxes and Deeming *et al.* (2019) showed that blue tits that were provided nest boxes of three different sizes, built larger and heavier nests in the boxes with greater base areas than conspecifics nesting in smaller boxes.

Our result that nestlings in the experimentally enlarged nests produced longer tails may indicate that another aspect of nestling condition (e.g. immunocompetence) that we did not measure was also affected by the experiment. Tomás *et al.* (2013) found that none of the measured breeding parameters (laying date, hatching date, clutch size, hatching success, brood size or fledging success) were affected by nest size manipulation in blue tits. However, the males in experimentally enlarged nests increased their risk-taking, which the authors postulated was due to the nest size functioning as a female sexual signal (Tomás *et al.* 2013). Because nest construction is costly (Mainwaring and Hartley 2009), nests can act as honest representations of the builders' fitness, which in turn may increase the parental investment provided by their mate (reviewed by Schaedelin and Taborsky 2009; Moreno 2012). In this study, however, we did not measure parental investment.

To conclude, our results do not provide strong support for the role of nest height in thermoregulation. Our finding that the nestlings' tail lengths were increased in the larger nests shows that the experiment had an effect. In line with the existing body of research, this effect may have been a product of sexual signalling rather than a thermoregulatory benefit (e.g. Mainwaring *et al.* 2012; Moreno 2012; Schaedelin and Taborsky 2009; Tomás *et al.* 2013).

3.2 Nest size and design as a female trait

In chapter **II**, we studied heritability and between-individual consistency in nest construction. We measured the height of 888 nests over nine breeding seasons (2008-2017, excluding 2011) and scored the composition of 1010 nests over 10 breeding seasons (2008-2017). The nest composition of 648 females and 557 males was scored either once ($N_{females}$ = 421, N_{males} = 353) or in several breeding seasons. We found the nest size and composition to be repeatable traits in blue tit females (nest height: $r = 0.4$, $p < 0.001$; proportion of feathers: $r = 0.23$, $p < 0.001$), but not in males (nest height: $p = 0.15$, proportion of feathers: $p = 0.5$). Spatial – i.e. between nest box – variation was significant in both models ($p < 0.001$) but explained more of the variance in the model of nest height (12 %) than in proportion of feathers (5 %). Female and male age did not affect the nest height (female: Wald x2 = 4.3, $p = 0.12$; male: x2 = 1.9, $p = 0.6$) or the proportion of feathers (female: x2 = 4.9, $p = 0.18$; male: x2 = 3.9, $p = 0.26$).

We found evidence of modest heritability in nest composition (proportion of feathers). Partitioning of female-variance into additive genetic (heritability) and permanent environmental effects indicated that 13 % of the variation in nest components was heritable, albeit statistically non-significant ($p = 0.07$). Nest

height had a point estimate of 12 % heritability, but this too was statistically non-significant ($p = 0.15$).

Our results suggest that nest construction in blue tits is a sex-specific trait limited to females. Modest heritability combined with the potential to respond to selection restricted to only half of the population suggests that while there is adaptive potential, selective forces must be strong and consistent for adaptation to occur.

To the best of my knowledge, this study was the first to assess heritability of nest characteristics using pedigree-derived estimates in a wild bird population. In comparison to the parent–offspring regression, which was the method used in the study by Møller (2006) and O'Neill *et al.* (2018), the current method uses resemblance in nest characteristics across all relatives in the pedigree. The benefit of this method is that it is much less affected by cultural inheritance (i.e. individuals copying their natal nest) and provides thus more accurate estimates of heritability.

3.3 Nest traits as an extended phenotype syndrome

In chapter **III**, we applied the concept of behavioural syndrome, with its focus on individual consistency and between-individual correlations (Sih *et al.* 2004), on nest construction.

First, we investigated between-individual consistency in nest ornamentation behaviour. We found that feather nest ornamentation is an individually consistent trait ($r = 0.25$, $p = 0.009$) that is limited to females in our study population. This implies that either the males in this population do not provide feather ornaments or they do so inconsistently from year to year ($r = 0.04$, $p = 0.33$). Our results thus contrast to those of Sanz and García-Navas (2011) and García-Navas *et al.* (2013) who found that males are responsible for providing feather ornaments in a Spanish population of blue tits.

Second, we studied the relationship between nest ornamentation and the nest height and proportion of feathers in the nest (repeatable female traits, chapter **II**). We found that nest ornamentation covaried with the propensity to use feathers in the nest lining (r = 0.42, SE = 0.16) but not with the nest height (r = 0.41, SE = 0.83). The correlation between the number of nest ornaments and the proportion of feathers in the nest thus forms a nest behavioural syndrome. Because nests function as extended phenotypes of the builders (Schaedelin and Taborsky 2009) – defined as all the effects in the environment caused by a gene that is expressed as a non-bodily attribute of an individual (Dawkins 1982) – we call this an extended phenotype syndrome. Instead of direct observations of individual construction

behaviour required to study behavioural syndromes, we used measurements of nest characteristics as surrogates for the behaviour.

Because correlated traits cannot evolve in isolation – assuming the correlation is genetically based – a syndrome in nest construction indicates that nest characteristics may have a restricted ability to adapt to environmental change. Our results indicate that to study nest traits in isolation will likely explain only a limited amount of the causal complexity behind variation in nest construction.

3.4 Fitness consequences of nest traits

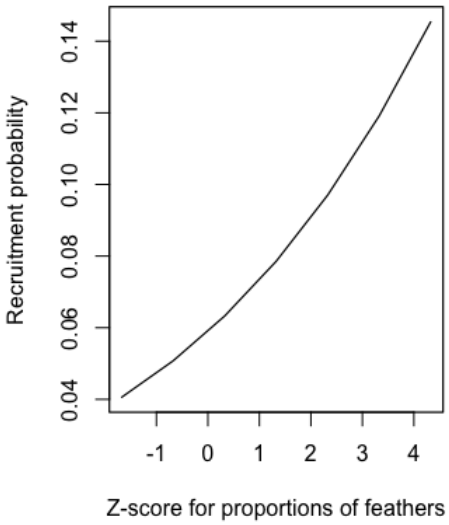

Figure 5 Fledgling recruitment probability as a factor of the proportion of feathers in the nest.

In chapter **IV**, we studied the fitness consequences of variation in the blue tit nest characteristics. We inspected the relationship between nest characteristics (the nest height, proportion of feathers in the nest and nest ornaments) and reproductive success (fledging and local recruitment probability). We found that the proportion of feathers in the nest had a significant (p = 0.006) positive effect on local recruitment probability (Fig. 5). Nest ornaments and lay date had negative, although statistically not significant, effects on recruitment (ornament, effect = -0.16. p = 0.10; lay date, effect = -0.18, p = 0.08). Female tarsus length and body mass (BM) were significantly related to fledging probability, however, with opposite effects (tarsus, effect = 0.46, $p < 0.001$; BM, effect = -0.24, p = 0.04). We also investigated the relationship between nest characteristics and nest ectoparasite (hen flea, *Ceratophyllus gallinae*) abundance but found none (nest height, effect = 0.02, p = 0.76; proportion of feathers, effect: 0.09, p = 0.17; nest ornament, effect = 0.06, p = 0.32).

The proportion of feathers in the nest had a considerable effect on local recruitment success and is thus related to female fitness. This is an exciting finding when combined with our previous results of heritability in the female propensity to use feathers in the nest (**II**). This thesis hence shows that variation in blue tit nest composition fulfills the two essential requirements for evolution. Variation in blue

tit nest construction has a consequence for fitness (i.e. is under selection, **IV**) and part of this variation is caused by genes (i.e. is heritable, **II**) and thus can evolve.

We found that the offspring of larger females (according to tarsus size) had an increased probability to survive the nestling period. Interestingly, females in better condition (according to the BM controlled for tarsus size) produced fewer fledglings. This somewhat curious result could perhaps be explained by the asset protection principle (Clark 1994). According to the hypothesis, females in better condition may have a higher chance of future reproduction (i.e. they possess larger current assets) and may be less willing to risk those assets by directing parental resources into current offspring over future reproduction.

To conclude, we found evidence of positive recruitment selection on a female nest trait: the proportion of feathers in the nest. The effect was maintained despite the fact that we statistically controlled for several other female characteristics (female age and condition), indicating the effect was more likely a product of the nest feather contents than individual quality. Curiously, we found no effect on nest traits affecting fledging success, which suggests that the selective benefit provided by the nest feather contents emerges sometime between fledging and recruitment to the breeding population.

4 Summary

In this thesis, I studied the causes and consequences of variation in blue tit nest construction. First, I investigated whether the size of the nest affects its capacity to aid in the thermoregulation of eggs and nestlings by means of experimentally enlarged and reduced nests (**I**). I found that the experimental manipulation was positively related to the nestling tail length. Because such effect is of minor biological importance, I conclude that our results provide little support for the thermoregulation hypothesis. According to the hypothesis, larger nests ought to provide thermoregulatory benefits. Most studies have found that more important than the mass of the nest base, which our experiment focused on, is the mass of the nest cup lining (e.g. Mainwaring and Hartley 2008; 2009; Deeming *et al.* 2012; Mainwaring *et al.* 2012). Nest height, in turn, may have a more important function in sexual signalling (Mainwaring *et al.* 2008; Moreno 2012; Tomás *et al.* 2013).

In the rest of the thesis (**II-IV**), I considered nest characteristics as individual traits with evolutionary potential. To the best of our knowledge, we are the first to provide evidence of heritability via pedigree-derived estimates of relatedness in combination with evidence of adaptive potential in bird nest construction. According to our main findings, the propensity to use feathers in the nest is a modestly heritable trait with fitness consequences (**IV**) and is thus subject to selection. However, the propensity to use feathers in the nest is limited to females in the population and as such its potential to respond to selection is halved (**II**). Therefore, for this trait to be able to evolve, it must be subjected to strong and consistent selection pressure. Moreover, the propensity to use feathers is correlated with nest ornamentation, indicating these two traits form an extended phenotype syndrome (**III)**. This is a term we presented to better describe a behavioural syndrome by proxy: instead of direct behavioural observations, we measured the physical remains of nest construction behaviour. This can have further implications on the evolutionary potential of blue tit nest construction – assuming the correlation is reflected on genetic level – because a syndrome may entail restricted flexibility in adaptation to change (**III).**

While this thesis does not provide direct evidence of female sexual signalling through nest construction per se – that would require further experimental

manipulation of nest material contents and recording of parental provisioning rates and nest defence behaviour - it does provide novel evidence of several ingredients that are required for sexual selection to evolve. First, we show that an aspect of nest construction is a repeatable and moderately heritable female trait (**II**). Second, we show that this trait is intercorrelated with another female nest trait and may reflect a correlation on a genetic level (**III**). Third, we show that the repeatable and heritable nest trait specific to females increases the probability of offpring recruitment to the breeding population (**IV**). These results combined with the existing body of research on sexual signalling via female built nests (e.g. Veiga and Polo 2011; Moreno 2012; Tomás *et al.* 2013) suggest that the tendency to use feathers in the nest may function as a secondary sexual signal of the female blue tit with the potential to evolve under sexual selection. It remains for the future studies to disentangle the connection between the nest feather contents and the probability of offspring survival to reproduce. Experimental manipulations of nest feather contents along with records of parental provisioning rates and risk-taking behaviour should be undertaken by future studies as well.

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